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Subunit Composition of the Crustacean Hemocyanins: Divergence in Incipient Speciation

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Abstract. The monomeric subunit composition of the oxygen carrier hemocyanin was examined in samples of the *Sesarma reticulatum* complex and of *Uca minax*, both of which are believed to be in the process of speciation. The samples were taken on the Atlantic and Gulf of Mexico coasts, from disjunct populations that are believed to have been isolated by the Florida peninsula since the retreat of the last glacier. In Atlantic and Gulf samples of the *S. reticulatum* complex, which is believed to be in the terminal stages of speciation, the hemocyanins differed qualitatively. Several electrophoretic bands found in one group, including an invariant band, were totally absent from the other. This difference exceeds that reported in a previous investigation of a variety of polymorphic allozymes in this species complex. It also exceeds the physiologically labile differences in hemocyanins found previously within a panmictic species of brachyuran crustaceans. In *U. minax*, which is believed to have diverged less, Atlantic and Gulf animals expressed the same number of electrophoretic bands at exactly the same positions. Nonetheless, highly significant differences in band frequencies distinguished both Atlantic samples from the Gulf sample, and somewhat less significant differences distinguished the Atlantic cold temperate zone samples from the warm temperate zone ones. The phenotypes of the major bands, defined as those present in high densities, qualitatively distinguished Atlantic from Gulf animals, but they did not differentiate the two Atlantic samples. The difference between Atlantic and Gulf members of this species also exceeds that found previously among polymorphic allozymes. These findings further support the hypothesis that the hemocyanins are among the first proteins to diverge structurally in brachyuran speciation.

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Introduction

With few exceptions, the crustacean hemocyanins (Hcs) are composed of many different polypeptide chains. When separated by charge in native polyacrylamide electrophoresis (PAGE), a dozen or more Cu-containing bands have been reported in some species (Mangum and Greaves, 1995; Mangum, 1996). In most species investigated thus far, the unusual heterogeneity provides the basis for intraspecific polymorphisms of a magnitude that is unprecedented in the literature on allozyme variation (Mangum, 1990, 1993a, 1996; Callicott and Mangum, 1993; Mangum and Greaves, 1996). In spite of these polymorphisms, the PAGE banding pattern appears to be species specific, even in sibling and previously cryptic species (Reese and Mangum, 1994; Mangum, 1996). The specificity suggests that the oxygen carrier diverges early in speciation, a hypothesis that is consistent with the quantitative importance of crustacean Hcs in aerobic metabolism (Truchot, 1992).

If the hypothesis is correct, one might expect to find differences between populations that are in the process of speciation. Below the species level, however, the available information on structural divergence of the crustacean Hcs appears to be contradictory. On the one hand, hybrid lobsters produced in the laboratory by unnatural matings of sibling, allopatric parental species can be readily distinguished from both parents (Mangum, 1993b). Less convincingly, because the comparison is based on different procedures used in different laboratories, samples from two subspecies of a fiddler crab differed so much that no bands were clearly shared by the two (Mangum and Greaves, 1996). On the other hand, no difference was found between the stone crab *Menippe*

mercenaria and the hybrid progeny of postulated natural matings between it and its sibling and former cryptor *M. adina* (Mangum, 1996). Also less convincing for the same reason described above, the phenotypes found in another pair of fiddler crab subspecies were quite similar (Mangum and Greaves, 1995).

In the present investigation, we analyzed the banding patterns of Hc monomers found in populations isolated by the Florida peninsula since the retreat of the last glacier. One pair is believed to be in the terminal stages of speciation, and the other group is believed to have diverged less. Because each sample proved to be polymorphic, we also characterized the intraspecific variation in detail, to ensure against confusing it with geographic divergence.

The species investigated are (1) the grapsid marsh crab *Sesarma reticulatum* (Say) from the Atlantic coast of North America and its sibling from the Gulf of Mexico, which has not yet been described and is currently known as *Sesarma* sp. (near *reticulatum*) (Zimmerman and Felder, 1991), and (2) the fiddler crab *Uca minax* (LeConte), also from both coasts.

Materials and Methods

Animals

All individuals investigated appeared to be intermolt crabs, as indicated by hard exoskeletons.

Atlantic populations of *Sesarma reticulatum* were sampled at Taskinas Creek and Indian Field Creek, both of which empty into the York River estuary in Virginia. Gulf of Mexico populations of *Sesarma* sp. were sampled at Cocodrie and Joseph's Harbor (Rockefeller refuge) in Louisiana, and at Ocean Springs in Mississippi. Atlantic cold temperate zone populations of *Uca minax* were sampled at five sites on the York River estuary (Ware Creek, Croaker Landing, York River State Park, Kings Creek, and Indian Field Creek) and one on the James River estuary (College Creek) in Virginia; these sampling sites are distributed along a salinity gradient ranging from oligohaline to upper mesohaline. A warm temperate zone population was sampled at Holden Beach in North Carolina. Gulf populations were sampled at Cypremort Point in Louisiana and Fort Bayou near Ocean Springs in Mississippi.

Preparation of material

Animals were bled immediately with a hypodermic syringe, and the blood was frozen until shortly before analysis. After thawing, serum was expressed from the clot by centrifugation, and an aliquot was diluted with a buffer (50 mmol l^{-1} Tris HCl, pH 8.9, containing 10 mmol l^{-1} EDTA) that dissociates Hc oligomers to their monomers and thus minimizes light scattering. Hc concentration was estimated from the absorbance of monomers, using the extinction coefficient for *Cancer borealis* Hc (Nickerson and van Holde, 1971), and additional aliquots of material were then diluted with the same buffer to produce a concentration of about 5 $\mu g\ \mu l^{-1}$. We emphasize the importance of the dissociation step in this procedure, which, in our experience, eliminates an error of about 15%.

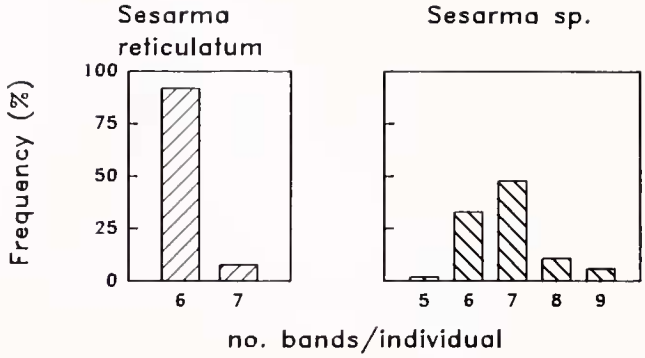

Figure 1. The frequency distribution of the total number of bands found in an individual of *Sesarma reticulatum* (n = 97) and *Sesarma* sp. (n = 64).

Electrophoresis

Native PAGE of dissociated material was performed at constant current, using the procedures and reagents detailed by Hames and Rickwood (1990). Two volumes (10 and 3 μl) of the material from each individual were examined. Most gels were stained only with Coomassie blue. To detect Cu, however, examples from each sample that collectively expressed all bands found in the sample were first electrophoresed, and then inspected on a long-wave UV transilluminator in the presence of bathocuproine sulfonate (Bruyninckx *et al.*, 1979). To estimate the proportions of the bands, examples were scanned with a BioRad Video Densitometer (Model 300A), and the scans were quantified with the 1D Analyst software package.

Results

Sesarma reticulatum *complex*

No difference was found between the individuals collected in Louisiana (n = 61) and Mississippi (3), and none was found between the individuals collected from the two tidal creeks in Virginia (n = 97). In both cases the data were combined for analysis and presentation. All bands were positive for Cu, and no band was related to sex.

Whereas all but a few individuals in the Atlantic sample expressed 6 Hc bands, the modal number in the Gulf

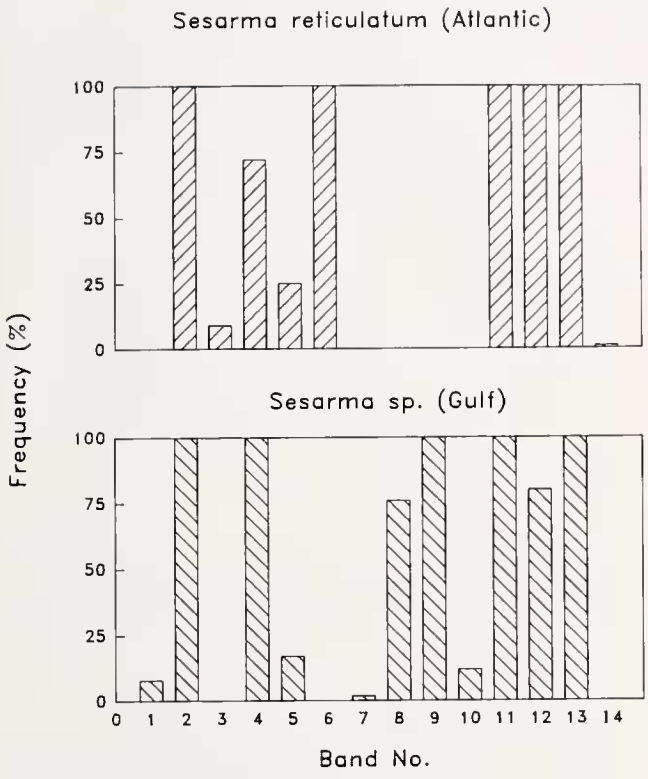

Figure 2. The frequencies of each band in *Sesarma reticulatum* and *Sesarma* sp. Sample size as in Fig. 1.

sample was 7 (Fig. 1). A total of only 9 bands was found in the Atlantic animals, but a total of 11 occurred in the Gulf animals (Figs. 2 and 3). Bands 2, 4, 5, and 11–13 in the two groups co-migrated to identical positions (Fig. 3). Two of these, however, differed in variability. Band 4 was invariant in *Sesarma* sp. but not in *S. reticulatum* (Fig. 2), in which it was often found in lower density as well (Fig. 3). Band 12 was variable in *Sesarma* sp. but not in *S. reticulatum* (Fig. 2).

In both groups a cathodic cluster of bands separated at some distance from an anodic cluster (Fig. 3). In both groups, the most common phenotype included bands in each cluster that co-migrated with bands in the other group, namely bands 2, 4, and 11–13. Nonetheless, the two groups were reliably distinguished by the presence in *Sesarma* sp. of a pair of both frequent and dense bands (8 and 9) in between the two clusters; because it was invariant, band 9 was an absolute marker. Members of this pair of bands were never found in *S. reticulatum* (Figs. 2 and 3). Additional, though less striking, differences include (1) one or two cathodic bands in each group that were not found in the other (1 in *Sesarma* sp. and 6 in *S. reticulatum*), and (2) two infrequent and less dense members (7 and 10) of the set of bands in between the cathodic and anodic clusters in *Sesarma* sp. Band 14 was found only once and only in trace densities in *Sesarma reticulatum;* it is unlikely to be a useful marker.

Both groups were polymorphic: 7 phenotypes were found in *S. reticulatum*, and 13 were found in *Sesarma* sp. (Table I). Of the 11 bands in *Sesarma* sp., 6 were variable, and the 5 qualitatively invariant bands still varied in density (Fig. 3). Although only 4 of the 9 bands were qualitatively variable in *S. reticulatum* (Fig. 2), all but one of the invariant bands varied in density (Fig. 3).

With the exception of the infrequent band 14, the variation in *S. reticulatum* was limited to bands 3, 4, and 5, which varied concomitantly in the classic pattern expected of the products of alleles encoded at the same lo-

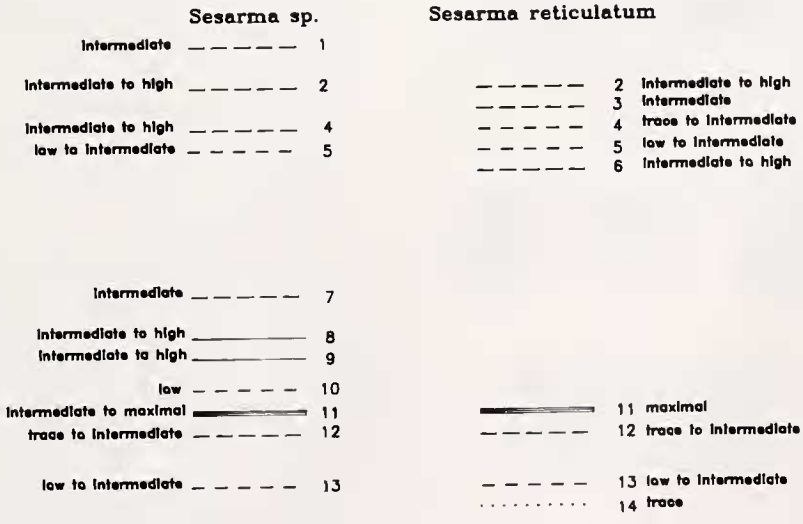

Figure 3. Diagrammatic representation of the electrophoretic bands in *Sesarma*. If no range is specified, then band density did not vary.

Table I

Phenotypes observed in Sesarma

A. *Sesarma reticulatum*															
		2		4		6					11	12	13		(70)
		2	3	4		6					11	12	13		(1)
		2		4		6					11	12	13	14	(1)
		2	3		5	6					11	12	13		(3)
		2		4	5	6					11	12	13		(3)
		2			5	6					11	12	13		(1)
		2	3			6					11	12	13		(19)
B. *Sesarma* sp.															
		2		4				8	9		11		13		(18)
		2		4				8	9		11	12	13		(41)
		2		4	5			8	9		11	12	13		(5)
		2		4	5				9		11	12	13		(3)
		2		4			7	8	9		11	12	13		(2)
		2		4					9	10	11	12	13		(2)
	1	2		4				8	9		11	12	13		(2)
	1	2		4	5			8	9		11	12	13		(5)
		2		4	5			8	9	10	11	12	13		(3)
		2		4				8	9	10	11	12	13		(5)
	1	2		4	5				9		11	12	13		(2)
		2		4					9		11	12	13		(15)
		2		4	5			8	9	10	11		13		(3)

The parenthetical number is the frequency (%).

cus. Each of these three bands was expressed either alone or together with one of the other two, but all three were never found together (Table I); nor were all three ever absent coincidentally. In general, density was highest when one of the three occurred alone (Fig. 4). Of the members of the triplet, band 4 was by far the most frequent (Fig. 2). In *Sesarma* sp., band 4 was invariant, and its density did not clearly decrease when band 5 was expressed. The third member of the *S. reticulatum* triplet (band 3) was never found in *Sesarma* sp.

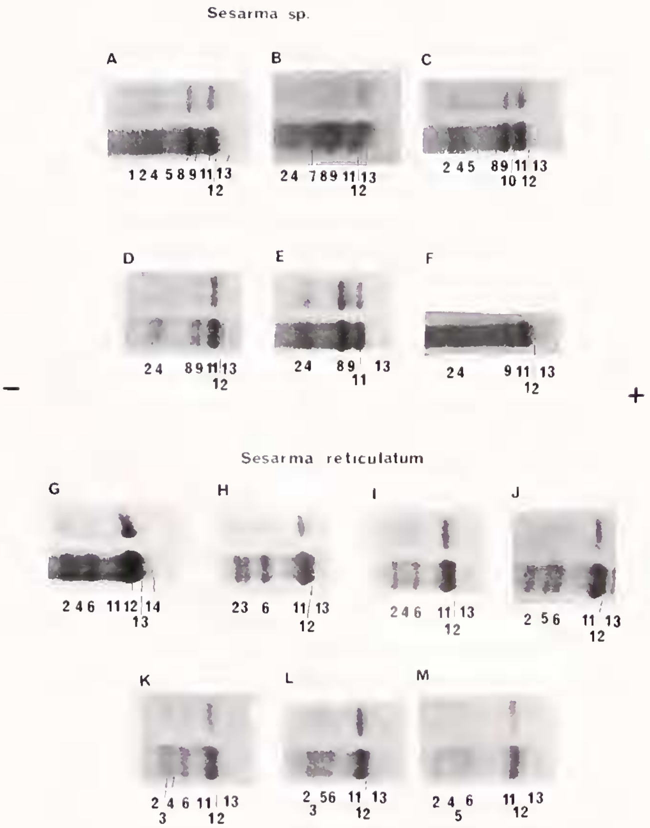

Figure 4. Examples of the variation in *Sesarma*. With the exception of F, each panel shows higher (bottom) and lower (top) concentrations of the material from the same individual.

In *Sesarma* sp., bands 8 and 12 also appeared to vary as expected of the products of a single locus. Again, each was found alone or together with the other, but one or both were always expressed. In this case, the variation in density (Fig. 3) appeared to be independent. The frequencies of each were quite similar in *Sesarma* sp., but band 8 was never found in *S. reticulatum* (Fig. 2).

Because this is the only of now several investigations in which we have encountered allozyme-like variation, we have chosen not to estimate "gene" frequencies and Hardy-Weinberg ratios until the genetic character of the observed variation can be corroborated. We do, however, report the frequencies of each phenotype in the *Sesarma* complex (Table I).

Uca minax

No difference was found between the Atlantic cold temperate zone animals ($n = 161$) collected at the several sites in Virginia; therefore, they are treated here as a single sample. Similarly, no difference was found between the 22 Gulf coast animals collected in Louisiana and the 49 collected in Mississippi, which are also treated here as a single sample. In each area, that is, Gulf coast and Atlantic cold (Virginia) temperate zone, a total of 17 bands were found. In any one sample, each band co-migrated to the same position as did a counterpart in the other two samples (Fig. 5). In other words, no marker bands were diagnostic of a particular sample. In addition, the modal number of bands expressed in each sample is the same (Fig. 6). Nonetheless, each sample differs significantly from the other two.

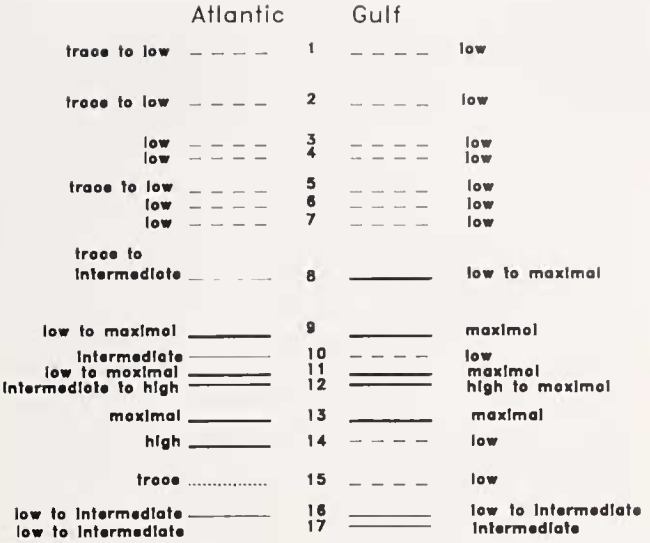

Figure 5. Diagrammatic representation of the electrophoretic bands in *Uca minax*. If no range is specified, then band density did not vary.

First, the frequencies of the bands differ (Fig. 7). Pairwise comparisons were made in a contingency test of independence. Because some cells in the Atlantic warm temperate zone sample were empty, the Chi-square statistic was used. Each of the two Atlantic samples differs from the Gulf sample at a probability level several orders of magnitude less than $P = 0.001$. Second, the variation in band density was similar in the two Atlantic samples, but it differed from that in the Gulf sample (Fig. 5). Perhaps most conspicuous, the phenotypes of the major bands, defined as those that comprise the majority of the material present, qualitatively distinguished the two Atlantic samples from the Gulf sample (Table II). Although the phenotypes of the major bands in the two Atlantic samples were the same, their frequencies differ significantly. Again, in a pairwise comparison by a contingency test of independence, the two differ at about $P = 0.001$.

Each of the three samples exhibited considerable polymorphism, with a very large number of phenotypes (Table III, Fig. 8). The sample from the Gulf coast, the smallest of the three ($n = 71$), had the smallest number of phenotypes (26). The sample from the warm temperate zone on the Atlantic coast, only slightly larger ($n = 73$), had a similar number of phenotypes (28). The sample from the cold temperate latitudes on the Atlantic coast, which was the largest ($n = 161$), exhibited a proportionately larger number of phenotypes (56), which is somewhat surprising. One would expect the rate of increase to diminish with increasing sample size.

In all three samples, the bands that occurred in either high densities or high frequencies were clearly positive for Cu. Bands 1, 2, 6, and 7 were neither clearly positive nor clearly negative because the combination of low density and low frequency precluded repeatedly overloading the gels for the less sensitive Cu detection procedure.

The only band that was invariant in all three samples was 14 (Fig. 8). However, bands 3, 4, and 13 were absent in only a very few individuals, and the variation in band 16 was confined to the Gulf sample (Fig. 7, Table III).

None of the qualitative variation of any one band was coupled to that of any other in a way that clearly suggests encoding at a common locus (Table III). One instance of coupled quantitative variation was noted: in the two Atlantic samples, the densities of bands 9 and 11 always varied concomitantly and directly.

Discussion

On the basis of allozymic variation at 13 polymorphic loci, Felder and Staton (1994) concluded that the genetic distance between Atlantic and Gulf of Mexico samples of *Sesarma reticulatum* (Say) is similar to that found in recently speciated populations of other brachyurans. The data strongly supported the authors' earlier conclusions, which were based on coloration, osmotic responses, and reproductive chronology (Zimmerman and Felder, 1991; Staton and Felder, 1992). All allozyme differences between samples of the two disjunct popula-

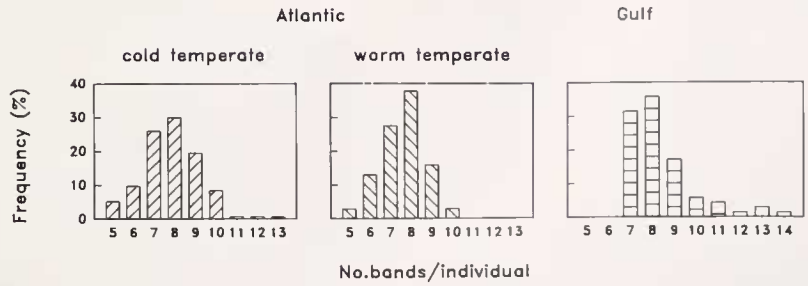

Figure 6. The frequency distribution of the total number of bands found in an individual of *Uca minax*. Values of *n* are 161 for the cold temperate zone sample, 73 for the warm temperate sample, and 71 for the Gulf sample.

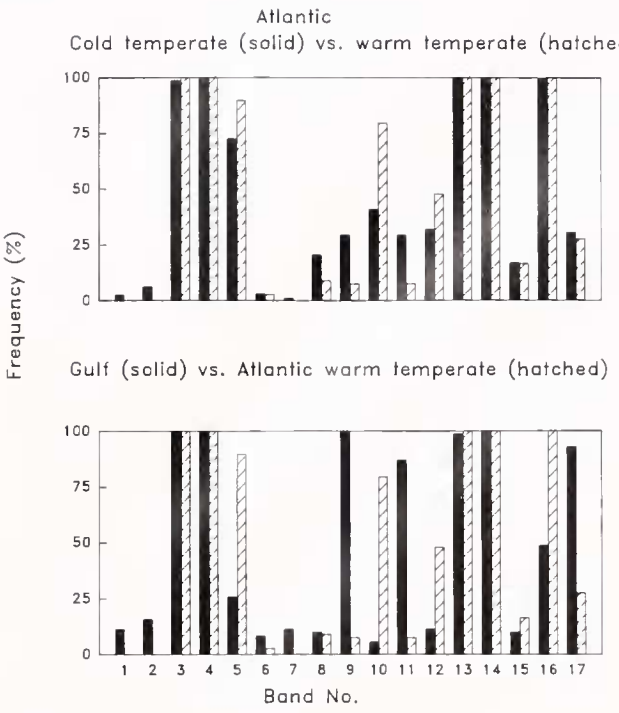

Figure 7. The frequencies of each band in *Uca minax*. Sample sizes as in Fig. 5.

tions, which they designated *S. reticulatum* and *Sesarma* sp., were quantitative, although several morphs completely absent from one coast existed in very high frequencies on the other.

The Hcs of the two *Sesarma* samples investigated here differ qualitatively. Band 9, found in all individuals of *Sesarma* sp., was totally absent from the *S. reticulatum* sample. Therefore, the present findings not only support the hypothesis of speciation, they also suggest that the Hcs differ more than the 13 polymorphic enzymes (much less an additional 7 enzymes, which were monomorphic in both species). This suggestion is constrained, however, by the use of different sampling sites on the Atlantic coast in the two investigations. Whereas Felder and Staton (1994) examined only warm temperate zone members of *S. reticulatum*, we examined only cold temperate zone representatives. Populations in the two biogeographic provinces on the Atlantic coast are by no means disjunct, but Cape Hatteras, which separates them, is a major faunal discontinuity (*e.g.*, Vernberg and Vernberg, 1972) and, in several taxa, populations found on either side of it differ (*e.g.*, *Uca minax* in the present investigation).

Felder and Staton (1994) reached different conclusions for *Uca minax*. In this case the allozyme variation was deemed insufficient to warrant specific separation. Although the inference was also based on 13 polymorphic loci, the variation at 9 of them was quite small. The only pertinent morphological evidence in this species is the intensity of the red coloration on the articles of the chelipeds and legs, which is strongly developed in Atlantic and western Gulf populations but, oddly, not in eastern Gulf populations. No comparative information on physiological or reproductive responses is available. As indicated above, our samples of eastern and western Gulf members of *U. minax* did not differ, indicating no relationship to the color patterns. Most of our sample originated from a Mississippi site where the color difference from Atlantic animals is maximal, and the remainder was collected in Louisiana, where the pattern more closely resembles that found in Atlantic members of the species. A similar conclusion was reached by Felder and Staton (1994), whose dendrogram based on allozymic differences does not reflect the color patterns.

Regardless, we still found clear, though primarily quantitative, differences in the overall banding patterns of the Hc monomers in each of the three samples analyzed. The same number of Cu-containing bands was found in Atlantic and Gulf animals, and each band in one sample co-migrated with a counterpart in the other. The most probable inference is that the co-migrants represent the same polypeptides. Nonetheless, the frequencies of the bands are very different in each sample. The overall differences between the two Atlantic samples are no greater than those observed within *Callinectes sapidus*, which are not genetically fixed in the adult stage (Mangum, 1990; deFur *et al.*, 1990). In contrast, the differences between either Atlantic sample and the Gulf sample are clearly greater than those in *C. sapidus*, which can be induced by environmental change. Although cross-acclimation experiments should be performed in

Table II

Frequencies of the phenotypes of the major bands, defined as those that comprised the majority of the total amount of material, in Uca minax.

	Atlantic		
	cold temperate	warm temperate	Gulf
	(% total)		
12 13 14	19.9	41.7	0
8 10 13 14	1.2	1.4	0
9 11 13 14	23.0	7.0	0
13 14	55.9	50.0	0
11 13	0	0	1.4
9 10 13	0	0	2.9
9 11 13	0	0	84.3
9 12 13	0	0	4.3
10 12 13	0	0	1.4
10 12 14	0	0	1.4
8 9 11 13	0	0	1.4
9 11 12 13	0	0	2.9

Table III

Phenotypes observed in Uca minax

A. Atlantic

1. Cold temperate

		3	4									13	14		16		
		3	4	5								13	14		16		
		3	4								12	13	14		16		
		3	4		6							13	14		16		
		3	4									13	14		16	17	
		3	4						10			13	14		16		
		3	4					9		11		13	14		16		
		3	4	5					10			13	14		16		
		3	4	5							12	13	14		16		
		3	4						10		12	13	14		16		
		3	4									13	14	15	16	17	
		3		5	6				10			13	14		16		
		3	4								12	13	14		16	17	
		3	4	5								13	14		16	17	
		3	4	5								13	14	15	16		
		3	4	5							12	13	14		16	17	
		3	4	5				9		11		13	14		16		
		3	4	5					10			13	14		16	17	
		3	4	5					10		12	13	14		16		
		3	4	5				9		11		13	14	15	16		
		3	4					9		11		13	14		16	17	
		3	4	5			8				12	13	14		16		
1	2						8	9		11		13	14		16		
	2	3	4	5								13	14		16	17	
		3	4	5								13	14	15	16	17	
		3	4	5			8		10			13	14		16		
		3	4					9		11	12	13	14		16		
	2	3	4					9		11		13	14		16		
		3	4	5			8					13	14	15	16		
		3	4	5				9			12	13	14		16	17	
		3	4	5			8					13	14		16	17	
		3	4	5				9		11		13	14	15	16		
		3	4	5					10		12	13	14		16	17	
		3	4	5			8	9		11		13	14		16		
		3	4		6			9		11		13	14		16	17	
		3	4				8		10		12	13	14	15	16		
		3	4	5					10			13	14	15	16	17	
		3	4	5	6			9		11		13	14		16		
		3	4	5			8		10			13	14		16		
		3	4	5			8		10			13	14		16	17	
		3	4	5				9		11		13	14		16	17	
		3	4	5			8		10		12	13	14		16		
		3	4	5			8	9			12	13	14		16		
		3	4					9		11		13	14	15	16	17	
	2	3	4	5			8	9		11		13	14		16		
1	2				6	7	8		10		12	13	14		16		
		3	4	5				9		11		13	14	15	16	17	
		3	4	5			8		10		12	13	14		16	17	
		3	4	5			8	9		11	12	13	14		16		
1		3	4	5			8		10			13	14		16	17	
		3	4	5					10		12	13	14	15	16	17	
		3	4	5			8	9		11		13	14		16	17	
		3	4	5			8		10			13	14	15	16	17	
		3	4	5			8		10		12	13	14	15	16		
		3	4	5		7	8		10			13	14		16	17	
		3	4	5			8		10		12	13	14	15	16	17	
1	2	3	4	5			8	9		11		13	14	15	16		

2. Warm temperate

		3	4									13	14		16		
		3	4								12	13	14		16		
		3	4						10			13	14		16		
		3	4									13	14		16	17	
		3	4	5								13	14		16		
		3	4						10		12	13	14		16		
		3	4	5					10			13	14		16		
		3	4	5							12	13	14		16		
		3	4	5								13	14	15		17	
		3	4	5								13	14		16	17	
		3	4								12	13	14		16	17	
		3	4	5			8		10			13	14		16		
		3	4	5					10		12	13	14		16		
		3	4	5				11	12	13	14		16				
		3	4	5				9		11		13	14		16		
		3	4						10		12	13	14		16	17	
		3	4	5							12	13	14		16	17	
		3	4	5					10			13	14	15	16		
		3	4	5					10			13	14		16	17	
		3	4	5					10			13	14	15	16	17	
		3	4	5					10		12	13	14	15	16		
		3	4	5					10		12	13	14		16	17	
		3	4					9		11		13	14	15	16	17	
		3	4	5				9		11		13	14		16	17	
		3	4	5			8		10		12	13	14		16		
		3	4		6			9		11		13	14		16	17	
		3	4	5			8		10		12	13	14		16	17	
		3	4	5	6		8		10			13	14		16	17	

B. Gulf

		3	4					9		11		13	14			17	
		3	4					9			12	13	14			17	
		3	4					9		11		13	14		16	17	
		3	4	5				9		11		13	14			17	
		3	4					9		11		13	14	15		17	
1		3	4				8	9		11		13	14			17	
		3	4					9		11	12	13	14			17	
		3	4	5				9			12	13	14			17	
		3	4					9		11	12	13	14		16	17	
		3	4	5				9		11		13	14		16	17	
	2	3	4					9		11		13	14		16	17	
1		3	4					9		11		13	14		16	17	
1	2	3	4					9		11		13	14			17	
		3	4	5		7		9		11		13	14			17	
		3	4	5		7		9		11		13	14		16		
		3	4	5		7		9	10			13	14			17	
		3	4	5			8	9			12	13	14			17	
		3	4		6		8	9		11		13	14	15	16		
1	2	3	4					9		11		13	14		16	17	
		3	4	5	6	7		9		11		13	14		16		
	2	3	4	5		7		9		11		13	14		16		
		3	4	5	6	7	8	9		11		13	14			17	
	2	3	4	5				9		11		13	14	15	16	17	
1	2	3	4					9		11	12	13	14	15	16	17	
	2	3	4	5	6		8	9			12	13	14	15	16	17	
1	2	3	4	5		7		9		11			14	15	16	17	

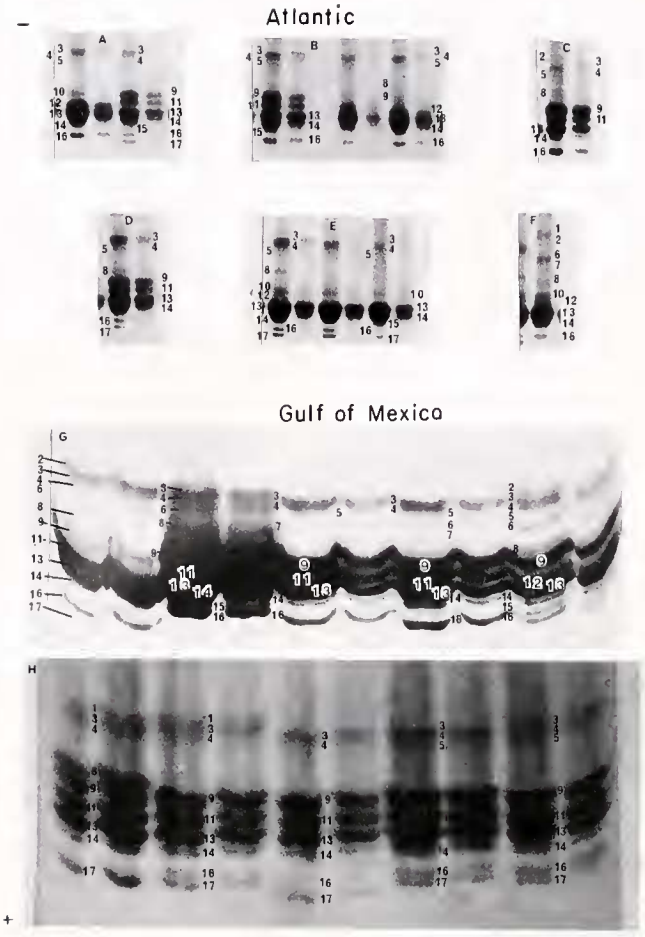

Figure 8. Examples of the variation in *Uca minax*. Top: Atlantic animals. With the exception of panel F, the lanes are arranged in pairs. Each pair of lanes, from left to right, shows a higher and a lower concentration of material from the same individual. Bottom: Gulf of Mexico animals. All lanes are arranged in pairs showing two concentrations of material from the same individual. With the exception of the pair at the far left, which shows first the lower and then the higher concentration, each pair alternates first the higher and then the lower concentration, as above.

U. minax, we suggest tentatively that the differences may reflect, at least in part, genetic divergence of the disjunct Atlantic and Gulf populations. This suggestion, if true, also supports the hypothesis that the Hcs diverge early in speciation.

We note that the differing magnitudes of the divergence in the two complexes examined here agree with the findings of Avise and co-workers as well as with those of Felder and students. On the basis of results for 19 species ranging from bivalves to chelicerates to teleosts, Avise (1992) found no evidence of a uniform molecular clock that measures the rate of divergence of Gulf and Atlantic populations.

We also suggest tentatively that very early divergence of the Hcs may be accelerated when the products of speciation must adapt to different thermal regimes (see Reese and Mangum, 1994), such as those found in the warm temperate (Gulf) and cold temperate (Atlantic, north of Cape Hatteras) zones.

Perhaps most important from a physiological point of view, the Atlantic and Gulf samples of *U. minax* are qualitatively differentiated by the phenotypes of the major bands, those that are most likely to influence respiratory properties. In addition, this inference remains valid when the comparison is restricted to Atlantic warm temperate zone *vs.* Gulf samples. The thermal regimes experienced by these two populations differ relatively little, certainly far less than the thermal regime experienced by either population and that experienced by Atlantic cold temperate zone populations.

Deevey (1950) suggested that environmental temperature is the primary factor responsible for the disjunction of Atlantic and Gulf species, a widespread phenomenon in a number of animal phyla (see also Avise, 1992). In

his scenario, disjunct populations are relicts of the retreat of the last glacier. The concomitant rise in temperature on both coasts around the Florida peninsula caused the retreat northwards of temperate zone species and their replacement in Florida by tropical and subtropical species invading from the south. Barnwell and Thurman (1984) correctly pointed out that biological factors such as the attendant changes in vegetation must also play a role in the disjunction of fiddler crab species. Whereas *U. minax*, for example, inhabits *Spartina* salt marshes, the species of *Uca* now found in subtropical regions of the Florida peninsula are mangrove dwellers.

Although we do not doubt the importance of habitat, we believe that temperature is likely to be extremely important in the present case. Temperature is the factor most often associated with divergence of respiratory properties among the Hcs of closely related species (Reese and Mangum, 1994). Consequently, the thermal sensitivities of the respiratory properties of Hcs expressing the most frequent phenotypes of major bands in *Uca minax* and the two *Sesarma* species will be of considerable interest.

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